PROJECT NUMBER: 1902
PROJECT TITLE: Tobacco Microbiology
PROJECT LEADER: D. J. Ayers
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PERIOD COVERED: October, 1988

I. SHREDDED STEM STUDY (FOR PROJECT ART)

A. **Objective**: To determine if pre-ART bright shredded stems with citrate spray (at 35 %OV) and post-ART "as is" stems (at 35 %OV) show a change in microbial numbers after 24 hours of storage at 35°C and to determine if post-ART dried stems (at 12 %OV) show a change in microbial numbers after 14 weeks of storage at 35°C.

B. **Results**: Bacterial counts did not increase, over initial counts, in the pre-ART stems with citrate and the post-ART stems at 24 hours of storage. A ~ 1 log increase, over initial counts, was seen at week 1 in post-ART dried stems but counts decreased below initial counts at 14 weeks of storage. Mold and yeast were within acceptable limits for the pre-ART and post-ART stems at week 14. Mold counts remained within acceptable limits but yeast counts exceeded acceptable limits at weeks 0 and 14 in post-ART dried stems.

C. **Plans**: Issue a memo reporting the results.

D. **Reference**:

1. Jones, J. Notebook No. 8590, pp. 59-61, 73.

II. YEAST ENCAPSULATED PEPPERMINT OIL (YEPO) STUDY

A. **Objective**: To test a sample of yeast encapsulated peppermint oil for viability and bacterial contaminants.

B. **Results**: There were no viable bacterial, mold or yeast cells/gram detected in the sample.

C. **Plans**: Issue a memo reporting the results.

D. **Reference**:

1. Gaines, O. Notebook 8690, pp. 53, 69-74.

III. BACTOMETER CALIBRATION CURVES

A. **Objective**: To generate calibration curves for determining the number of bacteria present in a sample using the Bactometer.

B. **Results**: Statistical analyses indicated that RL-TC, RL-150B, and and Burley can be run on the same calibration curve. Bright, Oriental, and Blend tobaccos require separate curves.

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C. **Plans**: Complete data collection for the completion of calibration curves for Bright, Oriental, and Blend.

D. **Reference**:

1. Chadick, D. Notebook 8625, pp. 158-161.

IV. DRY-PUFF CIGARETTE STUDY

A. **Objective**: To determine if the transmission of bacteria occurs after a cigarette is dry-puffed.

B. **Status**: Experiments were conducted using an aseptically designed apparatus. 1R4F cigarettes were dry-puffed, and the air passed through the cigarette was collected in sterile saline. The saline, dry-puffed and non-dry-puffed cigarettes were analyzed for bacterial growth.

C. **Results**: No bacterial growth was detected in the saline. The bacterial counts obtained from the dry-puffed cigarettes were similar to the counts from non-dry-puffed cigarettes.

D. **Plans**: No further experiments are planned unless requested.

E. **Reference**:

1. Chadick, D. Notebook 8625, pp. 150-153, 162.

V. ART FILLER STORAGE STUDY

A. **Objective**: To determine the effects of storage on the microbial load of ART filler at 35°C with 80% RH.

B. **Results**: Bacterial counts showed no increases over initial counts for the data accumulated from experiments 1-5 after 2-8 weeks of storage. The mold and yeast counts were variable and the majority of them exceeded acceptable laboratory limits ($\leq$ 80 mold and/or yeast colonies per gram, see Reference 1) in all five experiments to date (2).

C. **Plans**: Continue study.

D. **References**:

1. Crockett, E. A. The Microbial Quality Improvement Program (MQIP) As Conducted in the OC Semi-Works Primary Facility. Special Report #87-155; 1987 December 21.

2. Jones, J. Notebook 8590, pp. 73, 75, 77-80, 83.

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VI. CTB AND BURLEY SPRAYED BURLEY STORAGE STUDY

A. **Objective**: To determine if the addition of class tobacco dust (CT) to RBS would enhance microbial development on the Burley strip after 12 weeks of storage at 35 C with 80% RH.

B. **Results**: The storage study is complete through 4 weeks. The bacterial, mold and yeast counts are within the laboratory limits.

C. **Plans**: Continue study through 12 weeks of storage.

D. **Reference**:

1. Chadick, D. Notebook 8625, pp. 133-138, 141-142, 149, 154.

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